stabilizing their closed state. Furthermore, we have recently identified a series of residues in the C- and N-termini of Kir2.1 that are crucial for its sensitivity to cholesterol, suggesting a critical role for the cytosolic domain in cholesterol modulation of Kir channels.
Here we show that two cytosolic mutations, L222I and N251D, that are ~24Å apart from one another have similar effects on negating cholesterol and decreasing $PI(4,5)P_2$ sensitivity. Furthermore, both residues have similar effect on the open probability of the channel. These residues are allosterically coupled as the double mutant (L222I_N251D) reverts the effects of each single mutant. This result may be the major reason underlying the differences in cholesterol sensitivity and the strength of interaction with $PI(4,5)P_2$ of the four WT Kir2 channels, Kir2.1, Kir2.2, Kir2.3 and Kir2.4.
Moreover, our simulations suggest that the two residues are connected via two β strands through a critical salt bridge between K233 and D246. In agreement with our modeling results, the D246N mutation mimics each of the L222I and N251D mutations. This relationship demonstrates how the intricate arrangement of the cytsolic β sheets connects distant regions of the channel in a manner that enables control of channel gating and modulation.

129-Plat
Single Cell Dielectric Spectroscopy
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Pharmaceutical companies use a method known as patch clamping to quantify the potency of drugs by studying the effects of varied drug concentrations on membrane potentials. This particular method of patch clamping very accurately measures the effects and potency of pharmaceutical compounds as well as to study the range of side-effects caused by a drug. Patch clamping is a highly invasive and time consuming technique and has a high probability of destroying the cell. Dielectric spectroscopy is a non-invasive method that has acquired measurements comparable to those of patch clamping on a suspension of cells by placing them between a parallel plate capacitor and studying how the field changes with varying drug concentration. The field of microfluidics has brought about the possibility of scaling down this technique from a suspension of cells to single cells. A microfluidic flow chip was designed and fabricated to allow for cells in varied concentrations of drug solution to flow and pass through a parallel plate capacitor. Using numerical simulation, this capacitor was designed with guard electrodes to minimize fringing electric fields allowing for quick and accurate measurements comparable to patch clamp measurements.

130-Plat
Functional Assessment of Crystallization-Optimized G Protein-Coupled Receptors using Ion Channel-Coupled Receptors
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Ion Channel-Coupled Receptors (ICCRs) are artificial ligand-gated ion channels created by genetic fusion of G protein-coupled receptors (GPCRs) to a K+ inward rectifier channel (Kir6.2) such that the channel is a direct reporter of the receptor conformational changes. This concept has been validated with 4 prototypical GPCRs: the M2 muscarinic, the D2L dopaminergic, the β2 adrenergic and the opsin receptors (Moreau et al., Nature Nanotech 3:620 2008, Caro et al. PLoS ONE 6:e18226 2011, Caro et al. PLoS ONE 7:e43766 2012). Voltage-clamp recordings showed that ICCRs detect the agonist- and antagonist-bound states of the receptor via direct physical coupling. This GPCR-channel communication proceeds without any involvement of G proteins and the electrical signal amplitude is correlated with the ligand concentration.
The intrinsic instability of the GPCRs has proved a challenge to crystallographic studies. A successful approach, introduced in 2007 by Cherezov et al (Science. 318:1258) and subsequently applied to obtain 12 GPCR structures, consists in the insertion of the T4 phage lysozyme domain in the 3rd intracellular loop of the receptors. However, this modification abolishes G protein binding and prohibits related functional assays. Current characterization of crystallization-optimized GPCR(T4L) is performed by radiolabeled ligand assays or localized FRET techniques. Requiring no biochemical steps, ICCRs are an alternative tool to functionally characterize modified GPCRs that are unable to bind or activate G proteins and not amenable to most GPCR functional assays. We demonstrate here the validity of this tool with 3 different GPCRs (M2-muscarinic, β2-adrenergic and oxytocin receptors). The final application of this study would be the integration of this technology in the current crystallographic platforms dedicated to GPCR structure determination or for structure-function studies independent of G protein interaction.

131-Plat
High-Conductance K^+ Channels Show a Graded Sensitivity to Cell Volume Changes
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Slick (Slo2.1) and Slack (Slo2.2) Na^+/Cl^--activated high-conductance K^+ channels have been co-localized in CNS and are also probably present in the heart and epithelia. Furthermore, it has been found that these two channels can associate and form functional heteromers. A number of physiological processes, such as salt and water transport, central for neuronal activity, migration and apoptosis, involve changes in cell volume. We have previously shown that homomeric Slick channels are strongly regulated by changes in cell volume while the highly homologous channel Slack is totally insensitive. It is the aim of this work to evaluate if volume sensitive Slick subunits confer volume sensitivity to the otherwise insensitive Slack channels in tetrameric channels. For this purpose different configurations of Slick/Slack heteromeric channels were co-expressed with aquaporin1 in *Xenopus laevis* oocytes and cell volume changes of aprox. 5% were induced by exposure to hypotonic or hypertonic buffers. Whole-cell currents were measured by two electrode voltage clamp. Co-injections of Slick and Slack mRNA in different ratios (1:1-1:0.5-0.5:1) resulted in heteromeric channels sensitive to cell volume changes but to a smaller degree compared to homomeric Slick channels. Concatemeric Slick/Slack channels were also constructed and successfully expressed in oocytes. These chimeric channels showed, as co-injection experiments, higher whole cell currents than homomeric Slick or Slack channels and slower activation kinetics. Heteromeric channels resulting from the association Slick/Slack concatemers showed intermediate volume sensitivity between Slick and Slack channels, which seems to reflect the number of Slick subunits in the quatrameric channels. In conclusion, we have identified a heteromeric K^+ channel with graded sensitivity to small and fast changes in cell volume, a mechanism related with the number of volume sensitive subunits in the quatrameric channels.

132-Plat
Conformational Transition of KcsA Gating and the Mechanism of its pH-Dependence
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The bacterial K^+ channel KcsA has served as a prototypical system to study the architecture and the mechanism of gating of ion channels in response to proper stimuli. KcsA is activated in response to a drop in intracellular pH through the protonation of several His/Glu residues and the subsequent rearrangement of the helical bundle controlling the inner-gate opening. A constitutively open mutant channel (KcsA-OM) has been crystallized in several conformational states, each capturing a distinct degree of the inner-gate opening. However, due the heterogeneity of the structures and the use of antibodies, no firm conclusion could be made on the physiological relevance of the open conformation. In addition, due to the elimination of several charged residues, the mechanism of pH gating could not be fully determined by this particular mutant.
We have conducted both equilibrium and nonequilibrium driven MD simulations of the channel in membrane to probe the conformational variability of KcsA. When the pH-sensing residues are protonated in the simulations, the structure relaxes into an open conformation that resembles the crystal structure of KcsA-OM with second largest (23 Å) opening. The degree of opening captured in the simulations is consistent with that measured with EPR spectroscopy in the full-length KcsA, representing better the native state of the channel. Interestingly, the opening and closure of the cytoplasmic gate seem to be controlled by the competition between the protein-lipid interactions and several salt bridges between the channel's subunits. In particular, the neutralization of Glu118/Glu120 at low pH allows their entrance into the membrane, which permits the transmembrane helices surrounding the inner gate to tilt more and results in the opening of the channel.

133-Plat
Rate of Recovery from Slow Inactivation in K^+ Channels Controlled by Buried Water Molecules
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The transition of the selectivity filter of K+ channels between its two known important functional states, namely the conductive state and the slow